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MICROSCOPICAL NOTES

REGARDING THE

FUNGI PRESENT IN OPIUM BLIGHT.

BY

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(ON SPECIAL DUTY)

ATTACHED TO THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA.

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THE fungus forming the main subject of the following brief notes is one which has been so long known, and is of such common occurrence at home, that any details regarding the features presented by it in this country would be uncalled for were it not for its destructive effects on the opium crop in certain parts of India. Owing to the fact that in Western Europe poppies are either troublesome weeds or at utmost mere garden ornaments, it is quite natural that the existence of a fungus belonging to the same genus as the *Peronospora infestans* and bearing the same relation to the poppy which that far-famed parasite does to the potato, should have attracted little general attention and have been regarded as a mere matter of botanical interest. In this country, however, the case is very different, as any information regarding a parasite capable of exerting serious prejudicial influences on the growth of the opium poppy, acquires a practical importance from the value of the affected crop.

Peronospora arborescens: importance of its presence greater in this country than at home.

The observations on which these notes are founded were made during the current season on specimens for which I am indebted to Dr. King, who both provided blighted plants from the Botanical Gardens and procured others from Patna and from the gardens of the Agricultural and Horticultural Society here. They refer solely to the appearances presented by the parasite and the lesions resulting from its presence as they occur in the leaves, as,

Sources whence specimens were obtained for observation.

partly owing to difficulty of procuring perfectly fresh plants for study and partly to want of time, the examinations were almost entirely devoted to these points, and, even as regards them, are confessedly imperfect, experiments on the infection of previously healthy tissues not having been carried out for want of growing plants.

Peronospora arborescens constantly present on the blighted leaves.

All the specimens of blighted leaves, from whatever locality they were obtained, agreed in showing the presence of *Peronospora arborescens*,* a fungus which, as previously observed, is closely allied to that occurring in potato disease. The fungi belonging to this genus which, besides the two species mentioned above, includes various others affecting other host-plants, are true entophytes, their mycelium or vegetative apparatus being developed within the tissues of the plants which play the part of hosts, whilst their reproductive apparatus consists partly of filaments bearing the so-called spores or conidia which emerge from the substance of the leaves and project on the surface as a whitish mouldy layer, and partly of larger reproductive bodies termed *Oogonia* which are developed within the substance of the affected tissues. These consist of a sac containing a mass of living protoplasm, which subsequently forms the *Oospore*, and is ultimately invested with a thickened membrane, frequently marked with projecting tubercles, ridges or reticulations.† The former class of reproductive bodies, the conidia, are produced in extreme abundance and serve for the rapid diffusion of the parasite from plant to plant, whilst the latter, wrapped up in the tissues of the host and additionally protected by the thickened character of their envelopes, are supposed to be capable of retaining their

Reproductive apparatus in the Genus Peronospora.

* *Peronospora arborescens* is placed by De Bary in his synopsis of the *Peronosporei* under the section *Pleuroblastæ*. This section is defined as follows :—"Conidia non papillata, membrana circumcirca æquali hyalina aut violascente prædita, germinando tubum simplicem et aliquo superficiei puncto protrudentia." The *Pleuroblastæ* are further divided into four series, to the third of which, the "*Effusæ*," the present species is referred. The specific description is as follows :—"Stipites conidiophori graciles, elati, validi, superné 7-10-ies dichotomi, ramis plus minus flexuosis squarrosis patentibus sensim attenuatis, ultimis tenuissmis breviter subulatis plus minus arcuatis. Conidia parvula (diam. $\frac{1}{75}$—$\frac{1}{70}$ millim), subglobosa, membrana vix violascente. Oosporarum episporium brunneum." (*Du Développement des Champignons Parasites*, par M. A. De Bary. *Ann. Des. Sc. Nat. Quatrième Série Botanique*, T. XX, 1863, pp. 109-119.)

† The *Oospores* were discovered by Tulasne. The contents of the Oogonium are styled by De Bary "the gonosphere" until fertilization has been effected by means of the antheridia, and it is subsequent to this process that those changes take place resulting in the formation of the "*Oospore*" properly so called. De Bary op. cit. p. 17.

vitality under conditions destructive to that of the mycelium and conidia, and to be thus endowed with a capacity for germination after prolonged periods of rest. This process has, however, never been actually observed to occur in them, but is only inferred to exist from what has been observed in regard to the corresponding bodies in the allied genus *Cystopus*.* It is by means of such *Oospores* that the species of *Peronospora* affecting various plants at home are supposed to survive the winter and to be enabled to appear year after year.

Persistent mycelium in non-annual plants.

In plants which are not annual, but in which the entire plant or portions of it survive from one year to another, there is, moreover, another means by which such fungi are enabled to retain their vitality in a quiescent condition under adverse circumstances, until again exposed to those favorable to their development, even independent of the production of *Oogonia* and their contained *Oospores*. For example, the latter have never been detected on the *Peronospora infestans*, as that parasite exists in Europe on the potato plant, and yet it survives the rigours of the winter and appears year after year, because portions of its mycelium affecting the tissues of the tubers remain protected and dormant, wrapped up in the substance of the latter, and ready to grow and spread outwards with the young shoots arising from the tubers when they are planted.†

Relations of the fungi to the disease of the host-plants.

At one time there was a considerable amount of difference of opinion regarding the relations which the fungi of this genus bear to the diseased conditions of the host-plant with which their presence is associated. It was maintained by some observers that they were not the causes of these conditions, but that their development and diffusion was dependent on the previous existence of a diseased state, the destructive effects of which might be aggravated and hastened, but could not be originally induced, by the parasites. Later observations on this point, more especially those of Berkeley‡ and

* De Bary op. cit. p. 56.

† De Bary op. cit. p. 52. The author here instances *Peronospora infestans* and *Peronospora Ficariæ* affecting *Ficaria ranunculoides*.

‡ *Journ. Hort. Soc.*, Lond., Vol. I.

De Bary,* appear, however, clearly to prove that this is not the case, but that the diseased condition may be induced in healthy tissues by exposing them to the attacks of the parasites, and that the latter attack the healthy tissues and not those which have been previously injured or weakened by disease.

Hereditary predispositions in the host-plants.

Another question closely akin to this has of late attracted considerable attention in reference to the potato-disease:—Are particular breeds of the host-plant peculiarly liable to, or exempt from, the attacks of the parasite, or, in other words, is there such a thing as constitutional predisposition to the disease? The evidence on this point is as yet imperfect, but, so far as it goes, it is corroborative of a belief in the existence of such hereditary predispositions to attack or exemption, co-existent with perfect health in the host.†

Different appearances in blighted leaves.

Although, as previously mentioned, the presence of the parasite was detected in all the specimens of blighted leaves, the appearances due to its presence varied in different samples, due seemingly to a difference in the texture of the leaves; for, whilst in those of a thick nature the blight manifested itself in the form of black and slightly elevated patches, in thinner and more delicate specimens the affected spots appeared merely dried-up, brown, and withered.

On examining perfectly fresh specimens of the leaves obtained before sun-rise and before the dew had evaporated, nothing was to be found on their upper surfaces, but on the lower aspect they were covered with a forest of delicate erect filaments of the parasite bearing an abundant crop of conidia. The filaments are of such a size as to be readily detected with a low microscopic power, divide repeatedly in a dichotomous fashion, and bear large oval conidia at the extremities of their ultimate, more or less curved twigs. Both filaments and conidia are at this stage perfectly colourless. The filaments can be seen to issue from the stomata either solitarily or, more rarely, in pairs (Plate I, Fig. 1), and their presence is by no means limited to those

* Op. cit.

† *Side Lights on the Potato Disease*, by Worthington G. Smith, F. L. S. *Popular Science Review*, Ap. 1874, p. 150.

portions of the leaf which are manifestly blighted, as numerous specimens may be seen emerging from the surrounding textures, which as yet show no traces of discoloration or other evidence of the presence of the parasite appreciable to the unaided senses.

As the leaves wither and lose their freshness, whether from the destructive effects of the disease on the tissues, or merely as the result of drying, the filaments and spores lose their bright colourless appearance and become yellowish, and the latter especially ultimately assume a bright amber tint, but at no period do they exhibit any traces of the violet tinge so frequently present in the conidia of this genus, and which is said to be found in some degree in European specimens of the poppy *Peronospora*.

So long as the leaves are only beginning to be affected by the blight, the *Peronospora* is the only form of mould or fungus constantly present on them and extending with the extension of the disease, but, as will be noted further on, this is by no means the case in leaves which have been either partially or entirely withered by the progress of the blight, or which have been kept for any considerable time previous to examination.

Distribution and description of the mycelium.

It is, then, only fresh and recently affected leaves that are capable of furnishing specimens affording any clear idea of the relations of the parasite to the diseased and healthy tissues, and, even in such leaves, it is by no means an easy task to obtain satisfactory demonstrations. Very great difficulty was encountered in tracing the mycelial filaments through the substance of the tissues, on account of the extreme delicacy of their walls, the soft molecular nature of their contents, and the manner in which they are, as it were, moulded to the irregularities of the intercellular spaces. Latterly, however, by means of the employment of carmine as a staining medium, excellent demonstrations were obtained in abundance. Fine sections, having been cut with a razor, were allowed to soak in the carmine solution for some hours, and were then washed and mounted for examination. The protoplasmic contents of the mycelium,—fertile filaments and spores,—absorbed the carmine with great readiness, whilst the cells and tissues of the leaves remained only slightly affected by it. The mycelium now appeared mapped

out through the substance of the cellular tissue and could be easily traced over considerable areas (Plate I, Fig. 5).

The filaments were, as previously mentioned, closely adapted to their containing intercellular spaces, exhibiting projections and dilatations corresponding with the irregularities in these, were devoid of septa, and anastomosed freely with one another. They were not observed to penetrate the cells of the host, either directly or indirectly by means of *haustoria*, but appeared to be confined to the exterior of the cells. In this the mycelium resembles that of *Peronospora infestans*, in which also penetration of the cells of the host and *haustoria* have been hardly ever detected. The cells in the centre of a blighted patch of leaf-tissue are shrunken and discoloured, their contents being reduced to brownish or yellowish granular particles, whilst further out they exhibit every gradation of degeneration until at the periphery, where the mycelial filaments may be seen ramifying among cells which are apparently quite healthy, with rounded outlines and normal contents.

It was only in rare instances that individual filaments could be traced continuously for any considerable distance, as, owing to their irregular distribution among the cells, they are of course extremely liable to be divided, and it is only accidentally that the plane of section coincides with that of the distribution of individual fibres. Still in some cases very good demonstrations were obtained, including a few in which the fertile filaments remained *in situ*, and the mycelium could be traced from the stomata through which the former emerged (Plate I, Fig. 1).

Description of the fertile filaments.

The fertile filaments are erect, strong, and persistent, many remaining recognisable adherent to dried fragments of leaves attached to dead plants or obtained from the neighbouring soil. When quite fresh, they are perfectly colourless and full of finely molecular protoplasm ; but, on keeping, the latter shrinks into granular masses of a bright yellow colour, and remains without further change for many months in dried specimens. They are generally more or less curved, and divide repeatedly in a dichotomous manner, the ultimate twigs being curved and pointed, and each bearing a large oval conidium at the extremity (Plate I, Figs. 2—4).

The conidia: their characters.

The conidia are smooth, oval, and full of molecular protoplasm. The extremity attached to the stem is frequently somewhat narrower than the other, so that the outline becomes more or less distinctly obovate. Their average measurements are about $\frac{1}{1100}$ of an inch in length by $\frac{1}{1500}$ of an inch in breadth.* When fresh, they, like the rest of the plant, are quite colourless, but the protoplasm rapidly shrinks, acquiring a bright amber tint at the same time, and the conidia then remain unchanged for many months, and may long be recognised on the dead and withered leaves, adhering to the persistent filaments, or more commonly aggregated in amber-coloured masses on the surfaces of the leaves. In rare instances, monstrous specimens of conidia are produced, in which the contents are divided into two distinct masses, whilst the cell wall is correspondingly constricted to a greater or less extent.

Rapidity with which the conidia lose their vitality.

The conidia appear very rapidly to lose the power of germinating. In several instances in which fresh specimens were sown in water, germination was observed to take place, the method, curiously enough, being in general somewhat different from any of those described by De Bary as characteristic of the genus. The protoplasm at first became divided into several, generally four, distinct masses, as though a formation of zoospores were about to occur, but in place of this each mass gave origin to a simple germinal tube (Plate I, Fig. 3). The latter elongated considerably, but remained undivided, and showed no further development in the water, and, owing to the want of fresh-growing plants, experiments on their behaviour towards the living tissues of the host could not be carried out.

Oogonia and Oospores not detected.

Prolonged and careful search was made for *Oogonia* and *Oospores*, but entirely without avail, for, although fresh leaves in every stage of the disease and withered ones which adhered to blighted plants or had fallen on the surrounding soil, as well as specimens of the soil itself, were carefully examined, in no instance could any such bodies be detected.

* The actual measurements in two cases were $\frac{1}{1125}$ and $\frac{1}{1143}$, by $\frac{1}{1875}$ and $\frac{1}{1833}$.

This absence of *Oospores*, however, may have been a peculiarity in the leaves examined, or may be dependent on their development being confined to some part or parts of the host not submitted to examination, such as the capsules, cotyledons, first-developed true leaves, &c.

Examination of stems of blighted plants.

A few specimens of the stems of blighted plants were examined, but nothing special was detected in them. None of them appeared to be affected to any extent by the *Peronospora*, although mouldy patches, due to the presence of other common fungi, were frequently present on their surfaces. Sections were made in a few cases, specially in specimens obtained from the Botanical Gardens, in which the stem appeared to have rotted through near the base, and in which the substance was marked by black spots and lines on transverse and longitudinal section. No *Oospores* could be detected, and the presence of mycelium was not recognised, although, as staining was not had recourse to, it may really have been present, though concealed by the surrounding tissues.

A careful examination of the black spots and streaks was also made, but with equally negative results, the appearances seeming to be due to a degeneration of the vascular bundles, and more especially of the spiral vessels, which were thickened and discoloured, and contained much brownish granular matter.

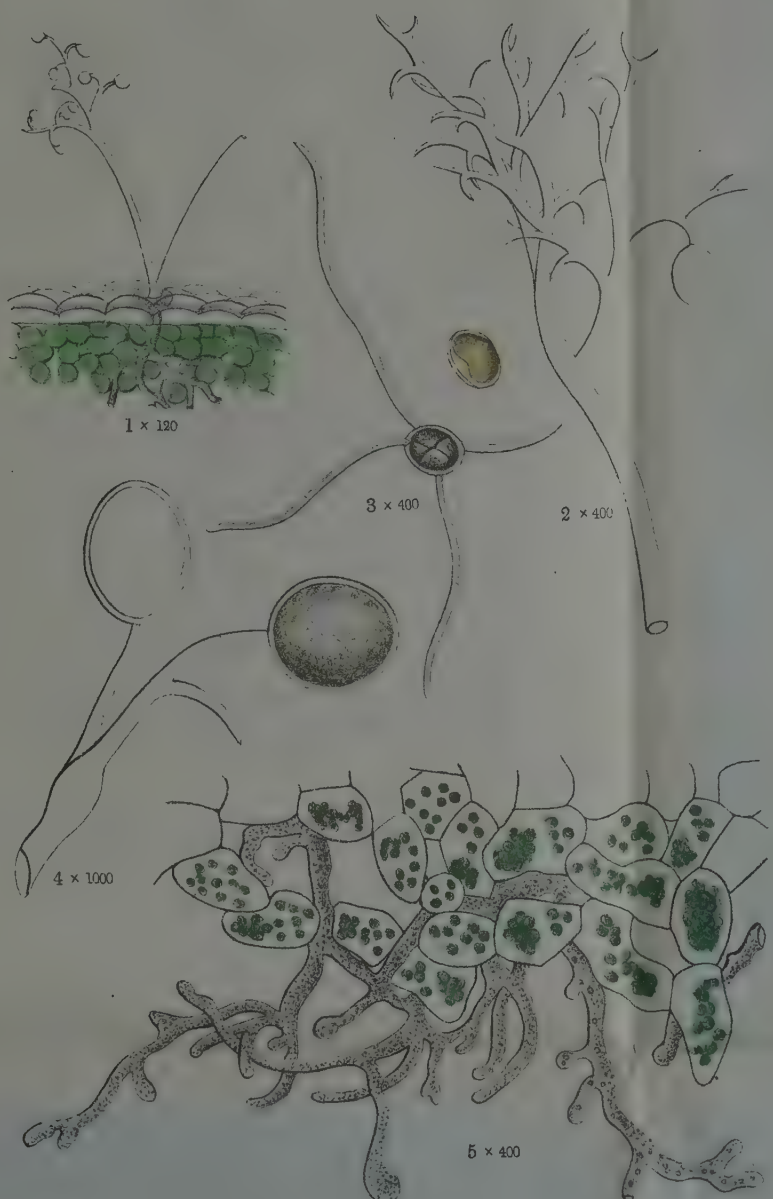
Argemone Mexicana: examination of.

Several specimens of the leaves of *Argemone Mexicana*, the common yellow Mexican poppy, were also examined in the hope of finding the *Oospores* of the *Peronospora*, but none of them showed any indications of the presence of the parasite.

EXPLANATION OF PLATE I.

Peronospora arborescens.

Fig. 1. Section through under-half of a poppy leaf, showing mycelium and fructifying filaments of *Peronospora*.
„ 2. Upper extremity of a fructifying filament, showing mode of branching and of attachment of spores.
„ 3. Spores : The upper one with shrunken protoplasm, the lower emitting germinal filaments.
„ 4. Ultimate twigs of a fructifying filament with spores attached.
„ 5. Mycelium ramifying amongst the cellular tissue of a leaf.



PERONOSPORA ARBORESCENS

Lithographed at the Surveyor General's Office, Calcutta, September 1874.

NATURAL HISTORY

The absence of *Oospores* in all the specimens submitted to examination is remarkable, as there can be little doubt that they must be produced, the recurrence of the disease from year to year being otherwise almost inexplicable, as the conidia are, in so far as observation goes, incapable of retaining their vitality when dried, or of germinating after any length of time. The possibility of their development being confined to special parts of the host has been already alluded to, and it is also possible that the germs of the mycelium may be contained in the seed. It is, of course, not necessary that the *Oospores* should be produced in any considerable numbers, for, if only a very few be produced and give rise to mycelium bearing the common conidia, the immense numbers of the latter produced, together with the ease with which they are disseminated and the rapidity of their germination under favourable circumstances, are amply sufficient to account for an extremely rapid diffusion of the disease when once originated in a locality. Even without the detection of the *Oospores*, however, there can be no doubt of the propriety of destroying the dead and withered leaves, stems, and other parts of blighted plants, and as much as possible preventing their remaining on or in the soil upon, or in the neighbourhood of, which poppies are to be again cultivated.

Oospores possibly confined to special parts of the plants.

The descriptions which have been hitherto given of the appearances presented by the blighted leaves refer only to those cases in which the disease is recent and in which the parasite is attacking the healthy tissues. Where the leaves have been affected for any time and have been partially or entirely deprived of vitality, or where they have been kept for some time previous to examination, quite a new series of phenomena are apt to occur, due to their invasion by various forms of fungi belonging to the multitudinous species so ready at all times to feed upon decomposing organic matter, and it is necessary that the effects which these produce should be carefully distinguished from those dependent on the growth of the parasite, which prepares the soil for them.

Appearance of moulds subsequent to the death of the leaves.

On examining specimens of the dead leaves, numerous dried stems and spores of the *Peronospora* will, as has been

already mentioned, be encountered; but they are in many cases obscured by a tangled mass of the mycelium and fertile filaments of various common forms of *Aspergillus* and *Penicillium*, together with greenish patches of *Cladosporium herbarum*. Various forms of the larger-spored *Dematiei* are also present, such as *Macrosporium*, *Helminthosporium*, *&c.* (Plate II, Figs. 7, 9, 10). Two of the commoner and at the same time most conspicuous forms consist of what appears to be a *Sporocybe* (Plate II, Figs. 5, 6), which will be again referred to farther on, and one bearing long-branched series of septate spores arising from a lax, creeping, almost colourless mycelium (Plate II, Fig. 8). These fungi are not like the *Peronospora* confined to one surface of the affected leaves, but appear indifferently on any dead portion of them.

Various species observed.

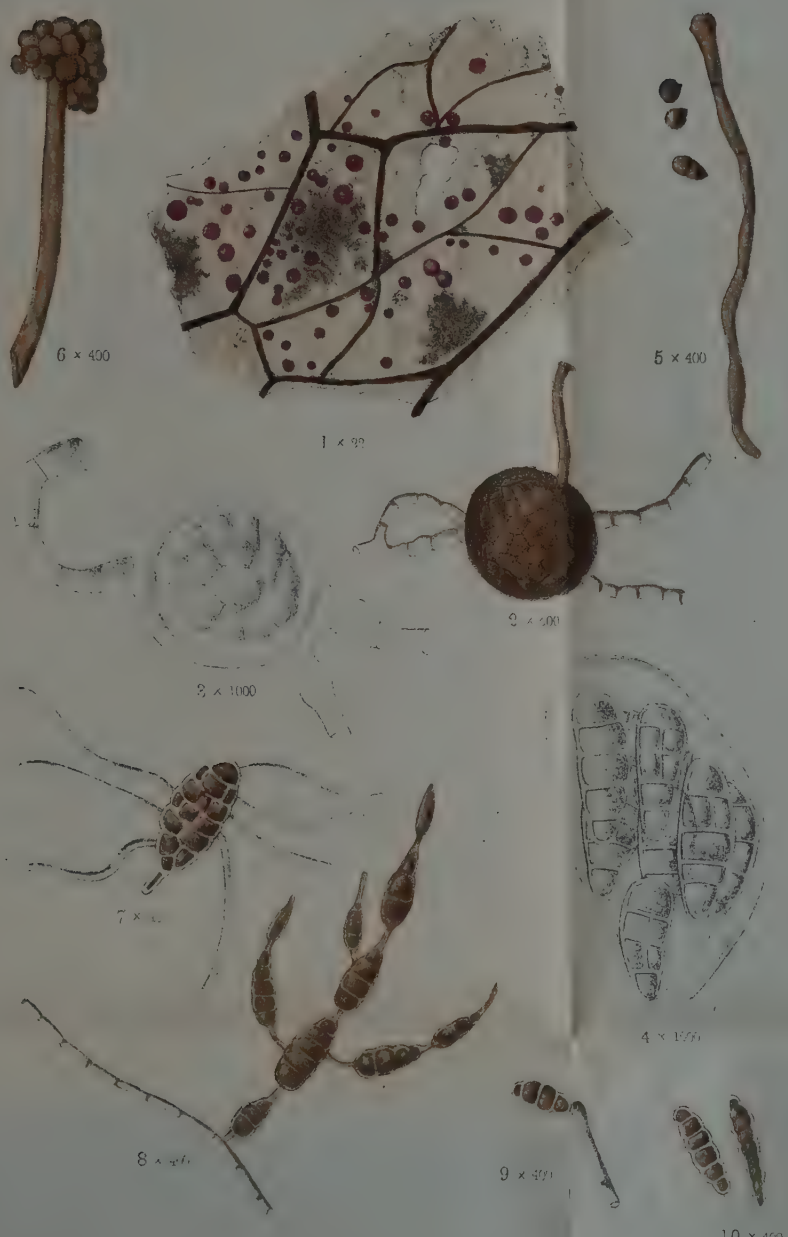
Beneath the layer formed by the filaments of the *Peronospora* and these other species, and appearing through the substance of the leaf, numerous brown cellular perithecia are visible (Plate II, Fig. 1). They arise from a mycelium of jointed filaments ramifying through the tissues of the leaf (Plate II, Fig. 2), and are more or less gregarious, occurring in the form of scattered patches. They are developed immediately beneath the epidermis of the upper surface of the affected leaf, through which the ostiolum ultimately opens, and are comparatively far removed from the lower surface of the leaf as a general rule. When fully formed, they are very distinct, of a dark brown colour, and contain two or three large asci, within which eight large, septate, somewhat curved sporidia are developed, which are at first colourless, but become

Perithecia developed in the blighted leaves.

EXPLANATION OF PLATE II.

Fungi from dead Poppy leaves.

Fig. 1. Low-power view of a portion of a poppy leaf with perithecia embedded in its substance.
„ 2. Mature perithecium with mycelial filaments and a portion of a conidiferous filament attached to it.
„ 3. Early condition of a similar perithecium.
„ 4. Ascus showing double walls and contained sporidia.
„ 5.
„ 6.
„ 7. } Various forms of fungal filaments and spores occurring on blighted
„ 8. } leaves.
„ 9.
„ 10.



FUNGI FROM DEAD POPPY LEAVES.

Lithographed at the Surveyor General's Office, Calcutta, September 1874

yellowish and ultimately deep brown (Plate II, Fig. 4). The size of the mature perithecia varies considerably, and their occurrence would hardly have merited farther notice here, had it not been that immature or partially abortive specimens might very readily be taken for *Oogonia* belonging to the *Peronospora*, more especially when, as is frequently the case, they are found in close association with the persistent stems of the latter. Owing to a confusion of this kind, a considerable amount of time was devoted to working out the relations and development of these bodies, and a short *résumé* of the results then obtained, is herewith subjoined for the benefit of others in this country who may hereafter take up the subject.

These may be mistaken for Oogonia and Oospores of the Peronospora.

The network of mycelium which bears the spores is formed of closely septate filaments, colourless or pale brown, ramifying through the substance of the leaf. As far as could be ascertained, the perithecia first made their appearance as saccular dilatations of these filaments to which others—antheridial filaments—were subsequently applied (Plate II, Fig. 3). The contents now became aggregated into a central mass which presently began to show a cellular structure, and, gradually increasing in size, came into contact with the walls of the sac, which ultimately become as it were moulded to the irregularities of the cellules of the contained mass. Within the latter, which now forms an endothecium, a development of asci now takes place. When fully developed, the perithecia vary considerably in size, the larger attaining a diameter of $\frac{1}{250}$ of an inch, whilst that of the smaller specimens ranges from $\frac{1}{500}$ to $\frac{1}{600}$ of an inch. As they mature, they press upwards against the cells of the epidermis, and when the latter give way, a small portion of the perithecium projects on the surface. An irregular ostiolum now makes its appearance in the exposed portion, revealing the contained asci and their large spores. The most remarkable feature in the asci is that they consist of two distinct sacs, one contained within the other and retaining the spores in a mass at some distance from the outer envelope (Plate II, Fig. 4). The spores are compound, exhibiting both transverse and longitudinal fission of the uniform protoplasmic mass of which they are at first formed. In many

Description of the development of these perithecia.

cases, however, the development of the perithecia is arrested; no asci are developed within the endothecium, but the contents remain as an amorphous mass of granular protoplasm. The cellular structure of the endothecium, moreover, is not so fully developed, and the outer layer of the perithecium remains more distinct, due to its not being pressed upon to the normal degree by the growth of its contents. It is in these cases that the perithecia are specially liable to be mistaken for *Oogonia* with their contained *Oospores*, for they then appear as sacs containing obscurely cellular masses full of granular protoplasm. The brown filaments of *Sporocybe* apparently arise from the same mycelium as these perithecia, and even in some cases from the latter themselves, and seem to be the conidial fructification of the same species of fungus.

Conclusion.

It is with great regret that I am obliged to bring this brief note on the microscopical aspects of the opium blight to a close without having been able to determine the localization of *Oospores*, or the existence of any other arrangement providing for the recurrence of the disease. I hope, however, that the next season may afford opportunities for the determination of these points, and in the meantime have thought it as well to bring forward what information I have on this important subject.

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